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The analysis of morphological differentiation of the epidermis of selected species of the genus *Epipactis* ZINN, 1757 (Orchidaceae: Neottieae)*

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ABSTRACT. Examining the qualities of epidermis can be useful in identification of some plant species. In an attempt to determine whether it can be useful in case of *Epipactis* ZINN, 1757: *E. helleborine* (L.) CRANTZ, *E. albensis* NOVÁKOVÁ et RYDLO, *E. atrorubens* (HOFFM.) BESSER, *E. palustris* (L.) CRANTZ, and *E. purpurata* SM. genera, their species have been studied. The following qualities, essential taxonomically, have been considered: the shape and size of epidermal cells; the presence or absence of subsidiary cells – a type of stomata; the presence, build and types of trichomes. The detailed studies proved that the identification of species of the *Epipactis* ZINN, 1757 genus based solely on the qualities of epidermis is not possible.

Key words: morphology, anatomy, epidermis, *Epipactis* ZINN, orchids

Abundant literature data (e.g. ELLIS 1979; LAWTON 1980; STACE 1993; BARUAH & NATH 1997) indicate that epidermis can constitute a good material for identifying a particular taxon thanks to its non-homogeneous structure. Examining the shape of epidermal, guard and subsidiary cells as well as the different types of trichomes may prove useful for identification of selected plant species. Regrettably, the quality of morphological features can vary depending on a taxonomic group, which makes it difficult to predict its value within the group without thorough examination (STACE 1993).

The object of the research was to verify whether the qualities of epidermis may be used in the process of identification of *Epipactis* ZINN, 1757 species, particularly when the examined material is taxonomically doubtful or incomplete, e.g. as a result of inappropriately secured herbarium sheets. It was also essential to determine possible differences between the species within epidermis qualities, which could be used in construction of determination key.

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MATERIALS AND METHODS

Analyses were conducted on herbarium material as well as that collected from the field in Poland, with the consent of the Polish Minister of Environment No.DOPog.-421-5/2002. The adaxial and abaxial epidermis of leaves was the main object of the examination. Small fragments of leaves of 5×5 mm size taken from the middle peripheral region of mature leaves were macerated in sodium hypochlorite for a period of two to four days. Both epidermal layers were stripped off gently from the mesophyll tissue with the help of a pointed needle and forceps (BARUAH & NATH 1997). The slides were examined under optical microscope and sketches were drawn. The following qualities of epidermis, important taxonomically, were taken into consideration: the shape and size of proper epidermal cells, the presence or absence of subsidiary cells – the type of stomata; the number of chloroplasts in stomata guard cells, the presence, the build and the types of trichomes. Optical microscope Nikon Eclipse 600 and scanning microscope LEO 435 VP were used in the examination.

RESULTS

Using available material, i.e. 169 specimens of *Epipactis helleborine* (L.) CRANTZ, 10 specimens of *Epipactis albensis* NOVÁKOVÁ et RYDLO and *Epipactis atrorubens* (HOFFM.) BESSER, 27 specimens of *Epipactis palustris* (L.) CRANTZ and 16 specimens of *Epipactis purpurata* SM., samples of adaxial (upper) and abaxial (lower) epidermis were made.

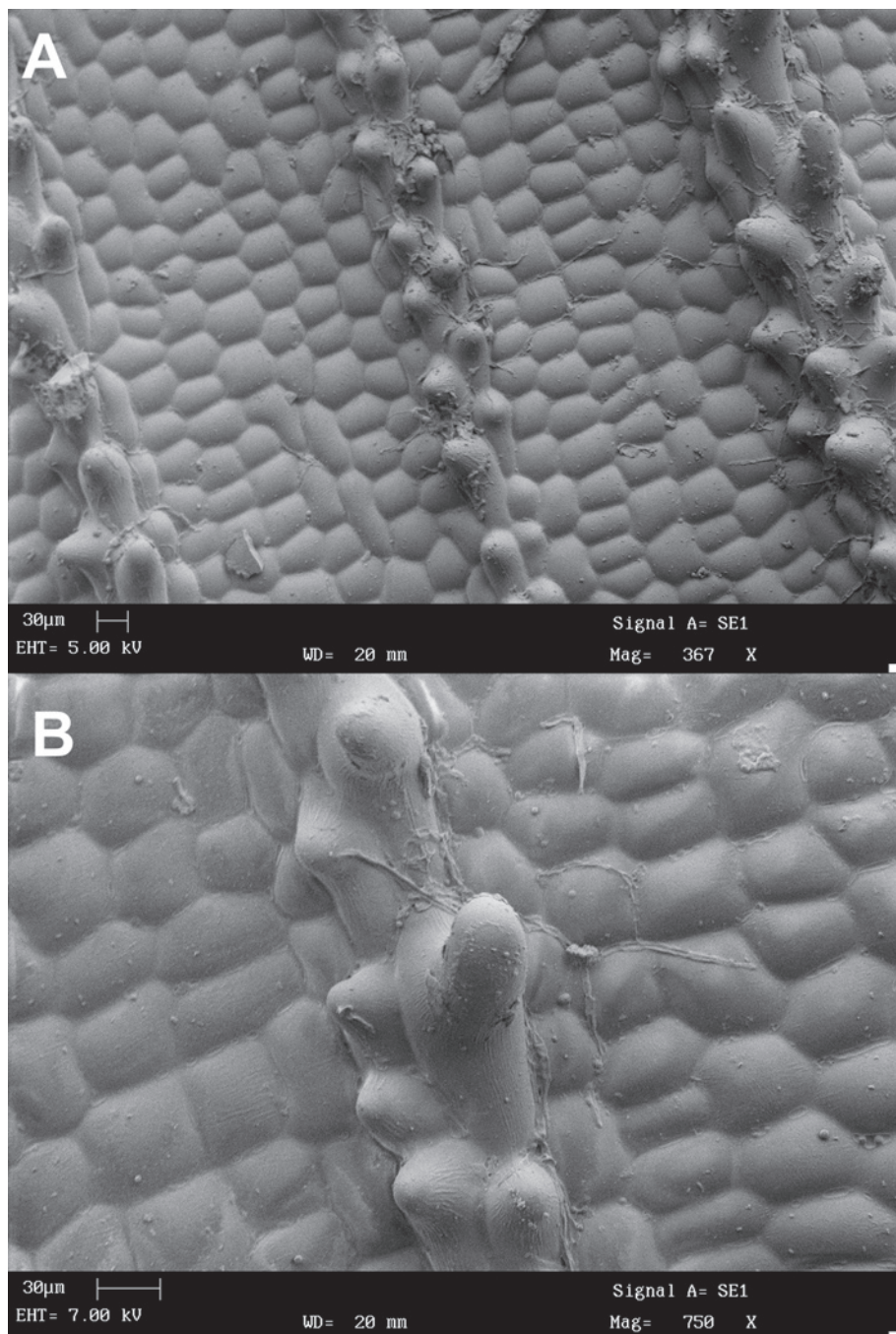
The number of chloroplasts in stomata guard cells is an indirect method for assessing the degree of ploidity, used successfully in cytotaxonomic examinations.

The numbers of chloroplasts in stomata guard cells were counted. The results are presented below (table 1):

Table 1.

The number of chloroplasts in stomata guard cells in the examined species of *Epipactis* ZINN, 1757 (Orchidaceae, Neottieae)

Species	Number of observations	Number of chloroplasts in stomata guard cells	Most often observed number of chloroplasts in stomata guard cells
<i>Epipactis helleborine</i> (L.) CRANTZ	1032	22,24,25,27,28,29,30,32,34,35,36,38	25 - 28
<i>Epipactis atrorubens</i> (HOFFM.) BESSER	610	21,22,23,24,25,26,27,28,29,31,32,33,34,37	24 - 28
<i>Epipactis palustris</i> (L.) CRANTZ	559	13,19,20,21,22,23,24,25,26,27,28,30,32,33	26 - 28
<i>Epipactis purpurata</i> SM.	562	12,15,19,20,24,25,26,27,28,29,30,31,32	27 - 29
<i>Epipactis albensis</i> NOVÁKOVÁ et RYDLO	496	20,21,22,23,24,25,26,27,28,31,32,35	26 - 29



Phot.1. Scanning electron micrograph surface view of the upper epidermis of *Epipactis helleborine* leaf with visible papillae (A, B)

The similar numbers of chloroplasts in a stomata guard cells obtained from representatives of different species do not allow to form long-range conclusions as the number of examined specimens was too small. The amount of examined material depended on its availability as well as the fact that the *Epipactis* genus is legally protected in Poland. Obtaining material from the field without the consent of the Minister of Environment is treated as an offence. Considering the small amount of specimens, it was not possible to make calculations which would confirm the statistic vitality of potential morphological-anatomic differences in the examined species.

The presence of trichomes on the upper and lower side of the leaf blade was found in all examined species within the genus. No fundamental interspecies differences in

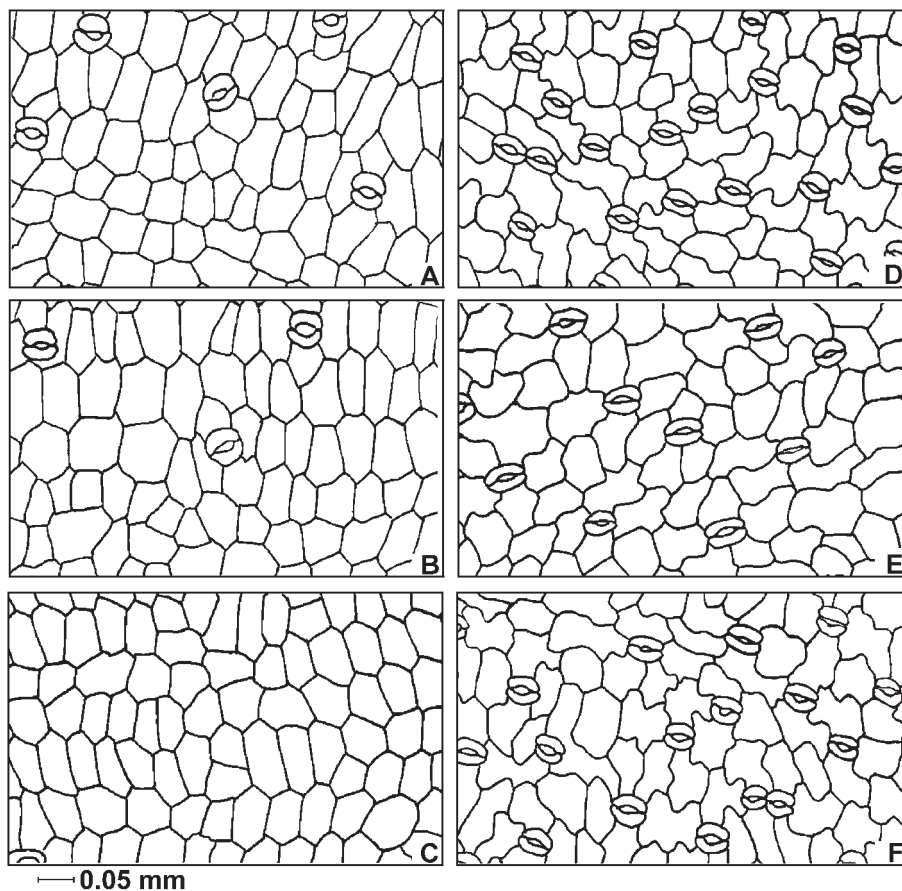


Fig. 1. Foliar epidermal structures of *Epipactis* ZINN, 1757 species: A - *Epipactis helleborine* (L.) CRANTZ, upper epidermis (Hb); B - *Epipactis palustris* (L.) CRANTZ, upper epidermis (Hb); C - *Epipactis albensis* NOVÁKOVÁ et RYDLO, upper epidermis (F); D - *Epipactis palustris* (L.) CRANTZ, lower epidermis (Hb); E - *Epipactis purpurata* SM., lower epidermis (Hb); F - *Epipactis albensis* NOVÁKOVÁ et RYDLO, lower epidermis (F); Abbreviations: Hb – slides made from the herbarium material; F – slides made from the freshly collected material

the shape or size of the trichomes, which could help in the process of taxa identification, were discovered (JAKUBSKA 2003).

An interesting cytological feature of all the species within the genus is the occurrence of papillae on the veins of the upper and lower side of the leaf blade (Phot. 1. A, B), whose role in the species of *Epipactis* genus has not yet been recognized.

The most vital among all the important anatomic features of the stomata is the way the epidermal cells surrounding the stoma, called subsidiary guard cells (STACE 1993), are arranged. However, the subsidiary guard cells did not occur in the examined species. No substantial differences between the species were found, nor were any regarding to the shape of the epidermal cells. On the basis of the examined material, STACE's (1993) suggestion that the qualities of stomata are not always an unfailing diagnostic criterion helpful in species identification was confirmed.

The study of the shape and size of the epidermal cells in the examined representatives of *Epipactis* genus does not allow a faultless identification as the shape of the cells in all the examined species is comparable (fig. 1).

Taking into consideration the samples prepared in the research, it was stated that, in the case of the species of *Epipactis* genus, herbarium material should not constitute the only source of information, but only complement the examinations carried out on freshly collected material, as the guard and epidermal cells might be deformed in the process of drying.

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